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Amended Claims

1. Retroviral expression vector containing at least the following elements in functional assembly:
a) DNA sequences for packaging of the vector RNA and for cell-specific expression of proteins or peptides encoded by heterologous DNA nucleotide sequences;
b) one or more DNA nucleotide sequences encoding a protein or peptide
wherein said DNA sequences for the cell-specific expression contain a cell-specifically controllable promoter region from a human endogenous retroviral DNA nucleotide sequence (HERV).

2. Expression vector according to claim 1 wherein said DNA sequences for cell-specific expression are derived from the LTR region and optionally from the non-translated region between the 5' LTR and the gag region of HERVs.

Subq1 3. Vector according to claim 1 or claim 2 wherein the whole LTR region, the U3 region, or the R and U3 regions are derived from a human endogenous retroviral nucleotide sequence.

4. Vector according to one or more of the preceding claims wherein said nucleotide sequences encoding one or more proteins or peptides are selected from one or more elements of the group consisting of marker genes, therapeutic genes, antiviral genes, anti-tumor genes, and cytokin genes.

5. Vector according to one or more of the preceding claims wherein said cell-specifically controllable promoter region is derived from the LTR region of a cell-specifically expressed endogenous human retroviral nucleotide sequence.

6. Vector according to one or more of the preceding claims wherein said human endogenous retroviral cell-specifically controllable promoter sequences are selected from one or more promoter sequences of HERV families of the group consisting of HERV-K, HERV-H, HERV-E, HERV-L, HERV-T, HERV-R, HERV-I, HERV-P, ERV9, HERV-W.

7. Vector according to one or more of the preceding claims wherein said promoter region besides the TATA box additionally comprises recognition and binding sites for regulatory proteins.

8. Vector according to claim 7 wherein said recognition and binding sites for regulatory proteins comprise the GC box, the CAAT box, enhancer sequences and repressor sequences as well as hormone responsive sequence motifs and wherein, optionally, additional recognition and binding sites for regulatory proteins from the LTR region of exogenous retroviruses and/or from cellular genes are comprised.

sub a2

9. Vector according to one or more of the preceding claims wherein said ~~vector~~ is a promoter conversion vector comprising a 5' LTR portion having the structure U3-R-

U5, one or more sequences selected from coding and non-coding sequences, and a 3' LTR portion comprising a U3 region which is partially or completely deleted wherein the deleted U3 portion is replaced by a cell-specifically controllable promoter region from a HERV LTR sequence, followed by the R-U5 region.

10. The mRNA or RNA of a retroviral expression vector according to one or more of the preceding claims.

11. Prokaryotic cell or eukaryotic cell containing a retroviral expression vector according to one or more of the preceding claims.

12. Eukaryotic cell containing a retroviral expression vector according to one or more of the preceding claims in an integrated form.

13. Use of a cell-specifically controllable promoter region from a human endogenous retroviral DNA nucleotide sequence for the regulation of the expression of foreign genes in retroviral expression vectors, preferably in ProCon vectors.

sub c3 14. Use of an expression vector according to one or more of the preceding claims for the expression of foreign genes in gene therapy.

15. Virion containing a retroviral expression vector RNA obtained by transcription of an expression vector DNA according to one or more of the preceding claims.

16. Method for the preparation of a virion according to claim 14 for the introduction of one or more nucleotide sequences encoding a protein or peptide wherein said retroviral expression vector according to one or more of the preceding claims is introduced into a suitable packaging cell line under such conditions that the virion is formed and released by the packaging cell line.

17. Method for the introduction of nucleotide sequences encoding one or more proteins or peptides into an eukaryotic cell wherein said cell is infected by a virion as defined in claim 14 under such conditions that the nucleotide sequences encoding the protein or peptide is inserted into the chromosomal DNA of the eukaryotic cell.

18. Method according to claim 17, wherein the eukaryotic cell is a mammalian cell.

19. Process according to claim 18, wherein the mammalian cell is a human cell.

Sub a4 20. Retroviral vector system comprising a ~~retroviral~~ expression vector according ~~to~~ one, or more of the preceding claims ~~and~~ a packaging cell line comprising at least ~~one~~ retroviral or recombinant retroviral construct encoding for the packaging proteins of the retroviral expression vector.

21. Retroviral vector system according to claim 20, wherein the packaging cell line comprises retroviral or recombinant retroviral constructs encoding for such retroviral proteins which are not encoded by the retroviral expression vector.

SUMMARY

The present invention relates to retroviral expression vectors with cell-specifically controllable promoters. For example, the vectors may be used for the cell-specific expression of genes of therapeutic value in the context of a gene therapy.

The present invention describes retroviral expression vectors containing at least the following elements in functional assembly:

a) DNA sequences for the packaging of the vector RNA and for cell-specific expression of proteins or peptides encoded by heterologous DNA nucleotide sequences;

b) one or more DNA nucleotide sequences encoding a protein or peptide wherein said DNA sequences for the cell-specific expression thereof contain a cell-specifically controllable promoter region from a human endogenous retroviral DNA nucleotide sequence (HERV).

P12088

Applicant: GSF-Forschungszentrum für Umwelt und Gesundheit GmbH

Title: Retroviral expression vectors on the basis of HERV LTR sequences

Description of the Figures of the PCT application

Tab. 1: Human endogenous retroviral elements with indication of the family, copy number and percentage in the genome

Tab. 2: Primers used for the amplification of different HERV LTR regions

Tab. 3: HERV LTRs analyzed
A: HERV LTRs from different cell lines and tissues indicating the homology
B: HERV LTRs published in the literature

Fig. 1: RT PCR strategy for the isolation of the U3/R regions of transcribed HERV sequences

Fig. 2g: Relative promoter activities of different HERV LTRs in various cell lines

Fig. 3a: Cell line plotted vs. relative promoter activity

Fig. 3b: Cell line plotted vs. relative promoter activity

Fig. 4: LTR R region modulated/promoter activity of HERV-K-T47D-related LTRs
A: Activation, cell line plotted vs. relative promoter activity
B: Inhibition, cell line plotted vs. relative promoter activity

Fig. 5: Sequences downstream of LTR R modulate the promoter activity of HERV-K-T47D-related LTRs
Cell line plotted vs. relative promoter activity

Fig. 6: Regulatory elements in the R region of HERV-K T47D LTRs

Fig. 7: Retroviral ProCon vectors pLESN-MMTV and pLESN-HERV-H

Fig. 8: Retroviral ProCon vectors pLX-MMTV and pLX-HERV-H

Fig. 9: a) Promoter conversion of ProCon hybrid vectors
b) Detection of correct promoter conversion by means of PCR and hybridization using a HERV-H and a psi probe

Fig. 10: a) Organization of the two ProCon vectors pLX-MMTV and pLX-HERV-H
b) Promoter activity of the HERV-H LTR as compared to the MMTV LTR following infection of CrfK cells